

# ABTS Cation scavenging activity and total phenolic content of three moss species

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## Abstract

A limited number of mosses have ever been studied in regard to antioxidant activity, with only a few belonging to the European species. As part of our ongoing screening of bryophytes as natural antioxidant resources, a relevant biological activity investigation was conducted on three moss species, namely *Brachythecium rutabulum* (Hedw.) Schimp. (Brachytheciaceae), *Calliergonella cuspidata* (Hedw.) Loeske (Hypnaceae) and *Hypnum mammillatum* (Brid.) Loeske (Hypnaceae), collected in Germany. The antioxidant activity of corresponding aqueous extracts was evaluated on the basis of their ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) cation scavenging activities. The total phenolic contents were determined as well. This study led to the detection of a moderate total phenolic content of the moss *B. rutabulum*, indicating for the first time its potential in searching for novel antioxidant agents.

**Keywords:** *Brachythecium rutabulum*; *Calliergonella cuspidata*; *Hypnum mammillatum*; antioxidant activity.

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There is abundant evidence that oxidative stress imposed by reactive oxygen species plays an important role in many chronic and degenerative diseases, such as atherosclerosis, ischemic heart disease, cancer, diabetes mellitus, neurodegenerative diseases and ageing [1,2]. Dietary supplements of antioxidants have become popular to enhance the body's antioxidant defenses. Natural antioxidants may come from vegetables, fruits and beverages [3–7]. On the other hand, synthetic antioxidants are widely used in the food industry to protect food from oxidation and spoiling. However, some of synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, have been found to be harmful for health due to their potential toxicity and carcinogenicity [8]. Therefore, there is an increasing interest for researchers in seeking for new resources of natural antioxidants, which are expected to be an alternative to synthetic ones [9,10].

Bryophytes (mosses, liverworts and hornworts) are the third largest group of the land plants after flowering plants and ferns, with approximately 15000 species spread worldwide. This group of plants possesses a strong antioxidative machinery which helps to cope up with extreme climates and stresses. Heavy metal, desiccation and ultraviolet radiation have been found to activate an array of different enzymes in bryophytes [11]. The study of antioxidant activity of the Antarctic

mosses *Sanionia uncinata* (Hedw.) Loeske and *Polytrichum alpinum* (Hedw.) G.L. Sm. var. *alpinum* has indicated their potential to be used as a possible source of antioxidants for medicinal and cosmetic purpose [12,13]. Moreover, the antioxidant activity of *Atrichum undulatum* (Hedw.) P. Beauv., *Polytrichum formosum* Hedw., *Pleurozium schreberi* (Brid.) Mitt. and *Thuidium tamariscinum* (Hedw.) Schimp. has been screened, and all tested species have showed antioxidant effects lower than the positive control, caffeic acid [14].

The objective of this study was to determine for the first time the antioxidant activity of aqueous extracts of three moss species, namely *Brachythecium rutabulum*, *Calliergonella cuspidata* and *Hypnum mammillatum*, in the context of their ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) cation scavenging activities and total phenolic contents.

## EXPERIMENTAL

### Plant material

All three samples of mosses were collected in Germany in December 2007: *Calliergonella cuspidata* (Hedw.) Loeske (Hypnaceae) and *Hypnum mammillatum* (Brid.) Loeske (Hypnaceae) in surrounding of Bonn and *Brachythecium rutabulum* (Hedw.) Schimp. (Brachytheciaceae) in Königsforst near Köln. Voucher specimens were deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia (bryophyte collection – BEOU No. 4704, No. 4709 and No. 4707, respectively).

## RESEARCH NOTE

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### Extraction procedure

The moss species were carefully selected and cleaned from soil and other contaminants. The gametophyte tips were used for the extractions. Air-dried parts of *B. rutabulum* (1 g), *C. cuspidata* (1 g) and *H. mammillatum* (1 g) were ground and extracted with hot water (3×20 mL) for 30 min. The extracts were filtered and concentrated by lyophilisation to give 0.11 g, 0.07 and 0.05 g of the residue (11, 7 and 5%, extract yields respectively), which were stored at 4 °C for further use.

### Screening of antioxidant activity

Determination of antioxidant activity was done following the ABTS method of Arnao *et al.* [15]. The reaction mixture contained 2 mM ABTS (2,2'-azino-bis(ethylbenzothiazoline-6-sulfonic acid)), 15 μM hydrogen peroxide and 0.25 μM horse radish peroxidase (HRP) in 50 mM phosphate buffer pH 7.5. The reactions were monitored at 730 nm (2501 PC Shimadzu, Kyoto, Japan) at 25 °C until a stable absorbance was obtained due to ABTS radical formation. Afterwards, different concentrations (0.05–0.80 mM) of ascorbic acid (AA) were added for a standard curve set-up. Addition of the extract in reaction mixture resulted in decrease in absorbance as a consequence of ABTS radical depletion. Absorbance alterations were read from standard curve and results were expressed as micrograms of AA equivalent per milligram of the extract (μg AA mg<sup>-1</sup> extract). The data are presented as mean ± standard error of the three samples analysed separately.

The total phenolic content in the extracts was determined according to the Folin–Ciocalteu's spectrophotometric (2501 PC Shimadzu, Kyoto, Japan) procedure using ferulic acid (FA) as a standard for the calib-

ration curve [16]. Samples were mixed with 0.25 N Folin–Ciocalteu reagents and after 3 min 0.2 M sodium carbonate solution was added and incubated for 60 min. Results were read at 724 nm and expressed as micrograms of FA equivalent per milligram of the extract (μg FA mg<sup>-1</sup> extract). The data are expressed as mean ± standard error of the three samples analysed separately.

### RESULTS AND DISCUSSION

The ABTS test showed the following data: 1 mg of *B. rutabulum* extract was equivalent to 14.37±1.3 μg of ascorbic acid (Figure 1); 1 mg of *C. cuspidata* extract was equivalent to 6.47±1.12 μg of ascorbic acid; 1 mg of *H. mammillatum* extract was equivalent to 0.74±0.02 μg of ascorbic acid. The total phenolic content in 1 mg of *B. rutabulum* extract was equivalent to 110.47±5.11 μg of ferulic acid as a standard (Figure 2); 1 mg of *C. cuspidata* extract was equivalent to 50.06±2.96 μg of ferulic acid; 1 mg of *H. mammillatum* extract was equivalent to 48.11±2.73 μg of ferulic acid.

Despite the majority previous studies [17,18], aqueous extracts are analysed in this screening, as it has been the case in recent works [19–21]. In comparison with the other two examined mosses, *B. rutabulum* had the highest total phenolic content. Our results indicate the potential of its extract in searching for novel antioxidant agents, which could be useful from food functional and drug medicine aspects. Hence, in the future, the phenolic compounds from this moss extract should be isolated, identified and explored for their nutritional values and health effects in relation to oxidative stress.

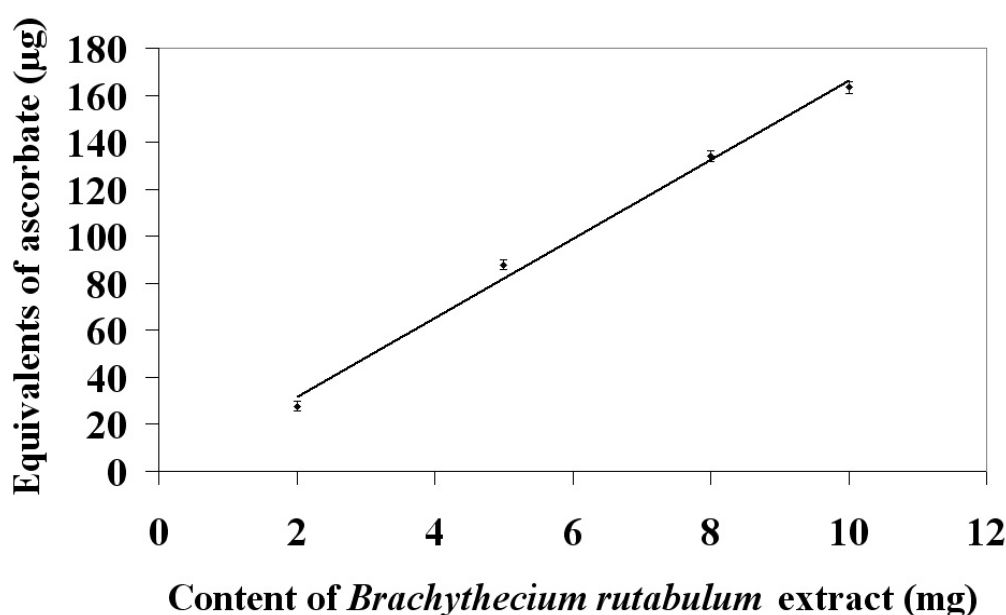


Figure 1. The ABTS cation scavenging activity of *Brachythecium rutabulum*.

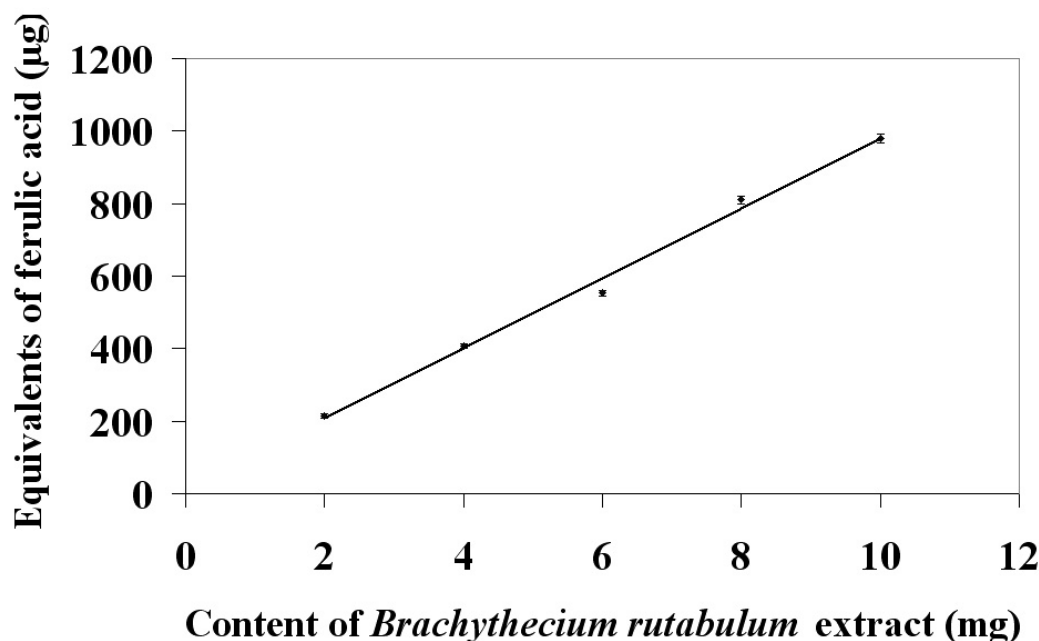


Figure 2. The total phenolic content of *Brachythecium rutabulum*.

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## IZVOD

### SPOSOBNOST NEUTRALIZACIJE ABTS RADIKAL-KATJONA I SADRŽAJ UKUPNIH FENOLA TRI VRSTE MAHOVINE

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(Kratko saopštenje)

Do sada je veoma malo proučavana antioksidativna aktivnost mahovina sa evropskog tla. Stoga je u ovom radu *in vitro* ispitivana sposobnost neutralizacije ABTS radikal katjona (2,2'-azinobis(3-etilbenzotiazolin-6-sulfonske kiseline)) i sadržaj ukupnih fenola vodenih ekstrakata tri vrste mahovine (*Brachythecium rutabulum* (Hedw.) Schimp. (Brachytheciaceae), *Calliergonella cuspidata* (Hedw.) Loeske (Hypnaceae) i *Hypnum mammillatum* (Brid.) Loeske (Hypnaceae)) sakupljene u Nemačkoj za vreme zimskog doba. Dobijeni rezultati ukazuju na to da ekstakt *B. rutabulum* može potencijalno da se smatra dobrim izvorom novih prirodnih supstanci sa antioksidativnom aktivnošću.

**Ključne reči:** *Brachythecium rutabulum* • *Calliergonella cuspidata* • *Hypnum mammillatum* • Antioksidativna aktivnost